

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification 6 :</b> <b>C12N 15/12, 15/62, 15/81, 9/12, 1/19,</b> <b>C12Q 1/68, C07K 14/47, 16/18, G01N</b> <b>33/50</b>	<b>A1</b>	<b>(11) International Publication Number: WO 97/44459</b> <b>(43) International Publication Date: 27 November 1997 (27.11.97)</b>
<b>(21) International Application Number: PCT/US97/09087</b> <b>(22) International Filing Date: 23 May 1997 (23.05.97)</b>  <b>(30) Priority Data:</b> 60/018,162                      23 May 1996 (23.05.96)                      US 60/019,462                      13 June 1996 (13.06.96)                      US  <b>(71) Applicant: CHIRON CORPORATION [US/US]; 4560 Horton</b> <b>Street, Emeryville, CA 94608-2916 (US).</b>  <b>(72) Inventors: CEN, Hui; 5142 Masonic Avenue, Oakland, CA</b> <b>94618 (US). WILLIAMS, Lewis; 3 Miraflores, Tiburon,</b> <b>CA 94920 (US).</b>  <b>(74) Agents: GUTH, Joseph, H. et al.; Chiron Corporation, Intel-</b> <b>lectual Property - R440, P.O. Box 8097, Emeryville, CA</b> <b>94662-8097 (US).</b>		<b>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR,</b> <b>BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE,</b> <b>GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,</b> <b>LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ,</b> <b>PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT,</b> <b>UA, UG, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD,</b> <b>SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU,</b> <b>TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI,</b> <b>FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent</b> <b>(BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD,</b> <b>TG).</b>  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i>
<b>(54) Title: GAMMA II ADAPTIN</b>  <b>(57) Abstract</b>  A mammalian protein involved in receptor-mediated endocytosis specifically binds to phosphatidylinositol 3-kinase. More specifically, it binds to the <i>Bcr</i> homology domain of the p85 subunit of phosphatidylinositol 3-kinase. Phosphatidylinositol 3-kinase also binds to platelet derived growth factor receptor. Other receptors may also bind, including those for insulin, insulin-like growth factor-1, colony stimulating factor 1, nerve growth factor, hepatocyte growth factor, stem cell growth factor, and epidermal growth factor. Mitogenesis is the consequence of the binding and internalization of these growth factors. Inhibition of the process of receptor-mediated internalization inhibits mitogenesis.		

BEST AVAILABLE COPY

## GAMMA II ADAPTIN

This application is a continuation-in-part of U.S. Serial No. 60/019,462 filed June 13, 1996, and U.S. Serial No. 60/018,162 filed May 23, 1996, the disclosures of which are expressly incorporated herein.

### 5 **BACKGROUND OF THE INVENTION**

Receptor-mediated endocytosis is the mechanism by which a variety of nutrients, hormones, and growth factors are specifically and efficiently transported into the cell as described in Goldstein *et al*, *Annu. Rev. Cell Biol.* 1:1-39 (1985). During this process, receptors are selectively concentrated in clathrin-coated pits from which they are rapidly internalized and delivered to endosomes; some  
10 receptors like the low density lipoprotein (LDL) receptor are constitutively clustered and internalized in the absence of ligand, while others, such as the epidermal growth factor (EGF) receptor are concentrated in the coated pits and internalized only after binding ligand, as described in Trowbridge, *Current Opin. in Cell Biol.* 3:634-641  
15 (1991). The protein complexes that link clathrin to transmembrane proteins are called adaptors. Plasma-membrane adaptors contain an  $\alpha$ -adaptin and a  $\beta$ -adaptin subunit, while adaptors found in the Golgi apparatus contain a  $\gamma$ -adaptin and a  $\beta'$ -adaptin subunit, as described in Robinson, *J. of Cell Biol.* 111: 2319-2326 (1990).

Despite these general outlines of the process of receptor-mediated  
20 endocytosis, the identities of all of the molecules involved and the interactions among them are not known. Thus, there is a continuing need in the art for identification of components of this important process.

### **SUMMARY OF THE INVENTION**

It is an object of the invention to provide a newly identified adaptin protein  
25 involved in receptor-mediated endocytosis.

protein, wherein said protein binds to a *Bcr* homology domain of a p85 subunit of phosphatidylinositol 3-kinase.

According to still another embodiment of the invention a method of screening for agents useful in inhibiting mitogenesis is provided. The method comprises the steps of:

contacting a test compound with a mammalian  $\gamma$ II-adaptin and a protein comprising a *Bcr* homology domain, wherein the protein binds to the  $\gamma$ II-adaptin in the absence of the test compound;

determining the amount of at least one of  $\gamma$ II-adaptin and the protein which is bound or unbound in the presence of the test compound, wherein a test compound which decreases the amount bound or increases the amount unbound, of at least one of  $\gamma$ II-adaptin and the protein is an agent useful for inhibiting mitogenesis.

According to still another aspect of the invention, a method of screening test compounds to identify agents useful for inhibiting mitogenesis is provided. The method comprises the steps of:

contacting a cell with a test compound, wherein the cell comprises:

a first fusion protein comprising (1) a DNA binding domain and (2) all or a portion of a mammalian  $\gamma$ II-adaptin, wherein the portion is capable of binding to p85 subunit of phosphatidylinositol 3-kinase;

a second fusion protein comprising (1) a transcriptional activating domain and (2) all or a portion of a p85 subunit of phosphatidylinositol 3-kinase, said portion comprising a *Bcr* homology domain of p85 subunit of phosphatidylinositol 3-kinase consisting of amino acids 134-322 as shown in SEQ ID NO: 4, wherein the interaction of the first and second fusion proteins reconstitutes a sequence-specific transcriptional activating factor; and

a reporter gene comprising a DNA sequence to which the DNA binding domain of the first fusion protein specifically binds; and

measuring the expression of the reporter gene, wherein a test compound that decreases the expression of the reporter gene is a potential inhibitor of mitogenesis.

According to still another aspect of the invention another method is provided for screening test compounds to identify agents useful for inhibiting mitogenesis. The method comprises the steps of:

contacting a cell with a test compound, wherein the cell comprises:

perinuclear region increases. This change in localization is consistent with a role in endocytosis.

Mammalian  $\gamma$ II adaptin protein as provided herein can be isolated and purified according to conventional techniques. Provided with the sequence of the protein, antibodies can be routinely generated against all or portions of it. Antibodies can be affinity purified, using the  $\gamma$ II adaptin protein or polypeptide portions thereof. Such antibodies can, in turn, be used to purify  $\gamma$ II adaptin from cell lysates, for example, or to quantitate the amount of  $\gamma$ II adaptin in cells. Techniques for immunizing host animals to raise antiserum are well known in the art. Similarly, techniques for using such immunized host animals to raise monoclonal antibodies are also well known. Both types of antibodies are contemplated within the scope of the invention.

Mammalian  $\gamma$ II adaptin proteins and polypeptides can also be made using recombinant host cells. The nucleotide sequence of one gene which encodes a mammalian  $\gamma$ II adaptin protein is provided in SEQ ID NO: 2. This gene, or other genes which are obtained from other mammals by hybridization under stringent conditions, or modifications of the gene which retain the coding capacity for a protein which binds to the *Bcr* homology domain of phosphatidylinositol 3-kinase, can all be used in recombinant cells to produce a  $\gamma$ II adaptin protein. Preferably such proteins will be at least 85%, and more preferably at least 90% identical to the sequence provided in SEQ ID NO: 1. Techniques for producing recombinant proteins given the nucleotide sequence encoding the protein are well known in the art. Selection of an appropriate technique is well within the purview of the skilled artisan. Portions of the gene can also be used to express portions of the  $\gamma$ II adaptin protein. These may be used, even if they do not retain the ability to bind to phosphatidylinositol 3-kinase, to raise antibodies, as discussed above. Typically a minimum number of contiguous amino acids to encode an epitope is 6, 8, or 10. However, more may be used, for example, at least 15, 25, 25, or 50, especially to form epitopes which involve non-contiguous residues.

adaplin are also useful as nucleotide probes for obtaining  $\gamma$ II adaplin variants and  $\gamma$ II adaplin genes from other species. Techniques for hybridizing polynucleotide probes and primers to obtain related family member genes are well known in the art.

5           Methods are provided for performing screening assays on agents which may be useful in inhibiting mitogenesis. The basis for all of these assays is the discovery of the binding interaction between  $\gamma$ II adaplin and phosphatidylinositol 3-kinase, and the functional consequence of the binding in mediation of the intracellular signaling of PGDF. Since PGDF is a known mitogen, agents which are found to inhibit the  
10           interaction of  $\gamma$ II adaplin and phosphatidylinositol 3-kinase will be useful in inhibiting mitogenesis.

          According to one method a  $\gamma$ II adaplin and a protein comprising a *Bcr* homology domain are incubated together in the presence of a test compound. In the absence of the test compound the protein binds to  $\gamma$ II adaplin. The amount of  
15           bound and/or unbound proteins is determined according to any technique known in the art, including any immunological technique. In order to facilitate the assay, one of the proteins may be bound to a solid support, or may be labeled with a radiolabel, or other detectable label. A useful agent is identified which decreases the amount of protein bound or increases the amount of protein unbound. The  
20           proteins can be prebound prior to the introduction of the test compound, or the test compound can be contacted with one of the two binding partners prior to incubation. The protein which has a *Bcr* homology domain may be, for example, phosphatidylinositol 3-kinase, rho-GAP, n-chimerin, p190, Bem2, Bem3, 3BP1, *Bcr*, or phosphatidylinositol 3-kinase. Alternatively, a protein containing the *Bcr*  
25           homology domain of one of the proteins can be used.

          A two-hybrid assay can also be used to assay for useful agents for inhibiting the interaction between  $\gamma$ II adaplin and phosphatidylinositol 3-kinase. According to such an assay, fusion proteins of each of the binding partners are used which each contain at least the domains necessary for the binding interaction. One of the  
30           binding partners is fused to a DNA binding domain and the other is fused to a

of the *Bcr* homology domain of the p85 subunit of PI 3 kinase cloned into pAS1-CYH, a GAL4 DNA-binding domain fusion vector. *Genes and Development* 7:555-569. The prey plasmid PACT encodes the GAL 4 transactivation domain. Upon reconstitution of the GAL4 transcription activating factor, *lacZ* and *HIS3* are induced. Transformants were selected for tryptophan prototrophy. Expression of the fusion protein was verified by western blotting with antibodies against the *Bcr* homology domain of the p85 subunit of PI 3 kinase. The resulting strain was confirmed by checking for its growth properties on SC-His plates containing 3-AT (3-aminotriazole, A8056, SIGMA, St. Louis, MO) and for its ability to activate the *LacZ* reporter. These tests were carried out relative to control strains carrying *SNF1* in the pAS1 vector. 3-AT concentrations of 25 mM to 50 mM are sufficient to select against pAS1 clones that fail to activate transcription on their own. A strain that failed to activate transcription was selected for transformation by the mouse thymus cDNA library.

A colony was used to inoculate 50 ml of SC-Trp and grown overnight at 30°C. An optical density reading (OD) of the culture at absorbance of 600 nm was taken and the culture was subsequently diluted to 0.1 (OD<sub>600</sub>) in 250 ml medium YEPD. [YEPD medium is made (for a one liter batch) with 10 g bacto-yeast extract, 20g bacto-peptone, 20 g glucose, and 40 mg adenine sulfate.] Synthetic complete minus Trp media is used to select for pAS1 but YEPD gave the best transformation efficiencies.

The cells were harvested at 5,000 rpm for 7 to 8 minutes in a small laptop centrifuge. The cells were washed once with TE (approximately 20 mls) and resuspended in 20 mls LiSORB and incubated at 30°C for 15 to 30 minutes. The cells were spun down as above and resuspended in 2 ml of LiSORB (LiSORB consists of 100 mM LiOAc, 10 mM Tris pH 8, 1 mM EDTA, and 1M Sorbitol), and an aliquot of 100 ul was placed into each of 20 tubes, and held on ice.

The DNA carrier mix was then prepared by boiling 200 ul of 20 mg/ml sheared salmon sperm DNA for 7 to 10 minutes. 800 ul LiSORB at room temperature was added and mixed by pipetting up and down. The mixture was

majority of false positives will not interact in this test. Some true positives may not activate either in this switch, so only a positive result is reliable. The pAS1 polylinker has been placed into pACT to facilitate this transfer, creating pACT2.

5 All general yeast protocols in this example are described in Methods in Enzymology Vol. 194 "Guide to Yeast Genetics and Molecular Biology" by Guthrie and Fink. Also Ausubel *et al* (1994) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, (Greene Publishing Associates and John Wiley & Sons, New York, NY), and Sambrook *et al.* (1989), MOLECULAR CLONING: A LABORATORY MANUAL, 2nd ed. (Cold Spring Harbor Press, Cold Spring Harbor, New York),  
10 are appropriate general references.

### Example 2

#### Generation of Polyclonal Antibodies In Sheep Against a Hydrophilic Peptide of $\gamma$ II Adaptin Protein

15 A 5.1 mg purified hydrophilic peptide of  $\gamma$  II adaptin protein was coupled through the terminal cysteine to diphtheria toxoid (DT) with the heterobifunctional cross-linking agent 6-maleimido-caproic acid N-hydroxysuccinimide ester (MCS) in the ration of 5.1 parts of peptide to 24.2 parts of DT (w/w).

20 The host selected for the immunization was a sheep Leicester/Merino cross, 3 to 5 years old. The immunization was conducted with a peptide-DT conjugate that was suspended in purified water at a concentration of 34.8 mg/ml, and emulsified by mixing with two volumes of Complete Freund's Adjuvant and injected intramuscularly, for a total volume of 1.0 ml (3.48 mg of conjugate, and 0.6 mg of peptide) per immunization. A second similar immunization followed  
25 2 weeks later, this time using Incomplete Freund's Adjuvant. A third immunization, a boost, was performed on week 12 using Incomplete Freund's Adjuvant.

The animals were bled from the jugular vein using a cannula. The blood was heated at 37°C for 30 minutes, chilled at 4°C for 15 hours and centrifuged.

activity against peptide

Sheep	prebleed	2nd bleed	4th bleed
2238	5 ml/112 titer	190 ml/882 titer	340 ml/1567 titer
2239	5 ml/68 titer	290 ml/459 titer	320 ml/1202 titer

5

activity against DT

Sheep	prebleed	2nd bleed	4th bleed
2238	5 ml/128 titer	190 ml/3232 titer	340 ml/16416 titer
2239	5 ml/ND titer	290 ml/5888 titer	320 ml/22976 titer

activity against Avidin

10

Sheep	prebleed	2nd bleed	4th bleed
2238	5 ml/99 titer	190 ml/98 titer	340 ml/280 titer
2239	5 ml/86 titer	290 ml/50 titer	320 ml/ 70 titer

**Example 3**

**Co-Immunoprecipitation of  $\gamma$  II Adaptin with P85 Subunit Indicates the  
Function of  $\gamma$  II Adaptin**

15

20

25

30

The C-terminal one third of  $\gamma$  II adaptin cDNA encodes an ear portion of  $\gamma$  II adaptin molecule (amino acids 518-791), which is thought to interact with phosphatidylinositol 3-kinase, as does full-length  $\gamma$ II adaptin. Therefore, myc-tagged ear  $\gamma$  II was transfected into Cos cells. Cos cells transfected with myc-tagged ear portion of  $\gamma$ II adaptin were cultured and lysed. (The myc tag is MEQKLISEEDL.) The cell lysate was immunoprecipitated with anti-p85 subunit of PI 3 kinase antibody. The precipitate was run on a reducing SDS-PAGE protein gel and transferred to a filter as described in Ausubel *et al* (1994) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, (Greene Publishing Associates and John Wiley & Sons, New York, NY), and Sambrook *et al.* (1989), MOLECULAR CLONING: A LABORATORY MANUAL, 2nd ed. (Cold Spring Harbor Press, Cold Spring Harbor, New York), for a Western hybridization. The filter was then blotted first with anti-myc monoclonal antibody to detect myc-tagged  $\gamma$  II adaptin and then blotted with alkaline phosphatase-conjugated anti-mouse antibodies, and then the filter was developed



## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(i) APPLICANT: Cen, Hui  
Williams, Lewis T.

(ii) TITLE OF INVENTION: Gamma II Adaptin

(iii) NUMBER OF SEQUENCES: 4

## (iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Chiron Corporation  
(B) STREET: 4560 Horton Street  
(C) CITY: Emeryville  
(D) STATE: California  
(E) COUNTRY: U.S.A.  
(F) ZIP: 94608

## (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

## (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:

## (viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Guth, Joseph H.  
(B) REGISTRATION NUMBER: 31,261  
(C) REFERENCE/DOCKET NUMBER: 1189-003

## (ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (510)923-3888  
(B) TELEFAX: (510) 655-3542

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 791 amino acids

195	200	205
Ala Leu Arg His Phe Arg Lys Val Val Pro Gln Leu Val Gln Ile Leu		
210	215	220
Arg Thr Leu Val Thr Thr Gly Tyr Ser Thr Glu His Ser Ile Ser Gly		
225	230	235 240
Val Ser Asp Pro Phe Leu Gln Val Gln Ile Leu Arg Leu Leu Arg Ile		
245	250	255
Leu Gly Arg Asn His Glu Glu Ser Ser Glu Thr Met Asn Asp Leu Leu		
260	265	270
Ala Gln Val Ala Thr Asn Thr Asp Thr Ser Arg Asn Ala Gly Asn Ala		
275	280	285
Val Leu Leu Glu Thr Val Leu Thr Ile Met Ala Ile His Ser Ala Ala		
290	295	300
Gly Leu Arg Val Leu Ala Val Asn Ile Leu Gly Arg Phe Leu Leu Asn		
305	310	315 320
Asn Asp Lys Asn Ile Arg Tyr Val Ala Leu Thr Ser Leu Leu Gln Leu		
325	330	335
Val Gln Ser Asp His Ser Ala Val Gln Arg His Arg Ser Thr Val Val		
340	345	350
Glu Cys Leu Gln Glu Thr Asp Ala Ser Leu Ser Arg Arg Ala Leu Glu		
355	360	365
Leu Ser Leu Ala Leu Val Asn Ser Ser Asn Val Arg Ala Met Met Gln		
370	375	380
Glu Leu Gln Ala Phe Leu Glu Ser Cys Pro Pro Asp Leu Arg Ala Asp		
385	390	395 400
Cys Ala Ser Gly Ile Leu Leu Ala Ala Glu Arg Phe Ala Pro Ser Lys		
405	410	415
Arg Trp His Ile Asp Thr Ile Leu His Val Leu Thr Thr Ala Gly Ala		
420	425	430

660	665	670
Ile Pro Ser Val Arg Val Phe Glu Arg Glu Gly Leu Gln Leu Asp Leu		
675	680	685
Ser Phe Met Arg Pro Leu Glu Thr Pro Ala Leu Leu Leu Val Thr Ala		
690	695	700
Thr Thr Thr Asn Ser Ser Lys Glu Asp Val Thr His Phe Val Cys Gln		
705	710	715 720
Ala Ala Val Pro Lys Ser Phe Gln Leu Gln Leu Gln Ala Pro Ser Gly		
725	730	735
Asn Thr Ile Pro Ala Gln Gly Gly Leu Pro Ile Thr Gln Val Phe Arg		
740	745	750
Ile Leu Asn Pro Asn Gln Ala Pro Leu Arg Leu Lys Leu Arg Leu Thr		
755	760	765
Tyr Asn His Ser Gly Gln Pro Val Gln Glu Ile Phe Glu Val Asp Asn		
770	775	780
Leu Pro Val Glu Thr Trp Gln		
785	790	

## (2) INFORMATION FOR SEQ ID NO:2:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2533 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TCGAGCTACG TCAGGGCCGA AGCCAGGATG GTGGTGCATT CGTTGAGACT TCAGGACCTA	60
ATCGAAGAGA TTCGCGGGGC CAAGACGCGAG GCCCAGGAAC GGGAGGTGAT CCAGAAGGAG	120
TGCGGCCCAA TTCGGGCCTC CTTCCGCGAT GGGGATCCCC TGCAGAGGCA TOGCCAGCTG	180
GCCAACTGTC TCTACGTCCA CATGTTGGGC TACCCGCCCC ACTTTGGACA GATGGAGTGC	240

AAGGTGCTGC AGTCCCATAT GTCCCTGCCA GCCACTCGGG GCTACGCCAT CACAGCCCTC 1620  
ATGAAGCTGA GCACCCGACT CCGGGGAGAC AACAAATCGTA TTCGCCAGGT GGTGTCCATC 1680  
TACGGGAGCT GTGTGGACTT AGAGCTGCAA CAGCGGGCTG TGGAGTATAA CACACTCTTC 1740  
CAGAAGTACG ACCACATGAG AGCCGCCATC CTAGAAAAGA TGCCTCTTGT AGAGCGTGGT 1800  
GACCCCCACG TTAAAGAGGG AGGGAAGGAG AAGCAAACGG AAGCCCAGCC CTTGGAAGTG 1860  
ACAGCCCCTG CCCCCACAGA ACCCCAGGCC ACCAACTCT TAGATCTACT GGATCTCCTG 1920  
GGTGACACTT CAGAGCCTCT CTCTTCTGGG CATGCCCAGC ATCTTCCTCC TCAGACTCCT 1980  
TCCCCAGGGG AAGCCTTAAT TCATCTCCTT GACCTTCCCT GTACACCGCC ACCCCCAGCT 2040  
CCCATCCCCA GTGTCAGAGT GTTTGAGCGT GAGGGCCTAC AGCTGGATCT TTCTTTCATG 2100  
CGGCCCTTGG AGACCCCTGC TTGCTCTTA GTCCTGCCA CCACCACCAA CTCCTCAAAG 2160  
GAGGATGTTA CCCACTTCGT TTGCCAGGCA GCTGTGCCCA AGAGTTTCCA GCTGCAGTTA 2220  
CAGGCCCCCA GTGGGAACAC AATTCCAGCT CAGGGTGGTC TTCCCATCAC CCAGGTCTTC 2280  
AGAATCCTCA ATCCTAACCA GGCACCTTTG CGACTTAAGC TGGCCTCAC CTACAACCAC 2340  
TCTGGCCAGC CAGTACAGGA GATCTTTGAG GTGGATAACT TGCCTGTGGA GACGTGGCAG 2400  
TAACCGACTG TGGTCAGTGT CTGGCCTGGG TGTCTCCAGG CTCCTGGTGT TCAAGGAACG 2460  
GAAATAAAGA CCCACGTAAA TGGCGAAGTA AACTTTATTT AAAGGCGACG TCAGGGCCCT 2520  
GACGTAGCTC GAG 2533

(2) INFORMATION FOR SEQ ID NO:3:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(11) MOLECULE TYPE: peptide

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ala Ser Ser Glu Asn Thr Glu His Leu Ile Lys Ile Ile Glu Ile Leu  
145 150 155 160

Ile Ser Thr Glu Trp Asn Glu Arg Gln Pro Ala Pro Ala Leu Pro Pro  
165 170 175

Lys Pro Pro Lys Pro Thr Thr Val Ala Asn Asn Gly Met  
180 185

12. The preparation of antibodies of claim 10 wherein the antibodies are monoclonal.

13. The preparation of antibodies of claim 10 wherein the antibodies are purified from an animal antiserum.

5 14. The preparation of antibodies of claim 10 wherein the antibodies are affinity purified.

15. A subgenomic polynucleotide which encodes a mammalian  $\gamma$ II-adaptin protein, wherein said protein binds to a *Bcr* homology domain of a p85 subunit of phosphatidylinositol 3-kinase.

10 16. The polynucleotide of claim 15 which encodes a mammalian  $\gamma$ II-adaptin protein as shown in SEQ ID NO:1.

17. The subgenomic polynucleotide of claim 15 which is intron-free.

18. The subgenomic polynucleotide of claim 15 which comprises the sequence shown in SEQ ID NO:2.

15 19. A vector comprising the polynucleotide of claim 15.

20. A vector comprising the polynucleotide of claim 16.

21. A vector comprising the polynucleotide of claim 17.

22. A vector comprising the polynucleotide of claim 18.

23. A recombinant host cell which comprises the vector of claim 19.

20 24. A recombinant host cell which comprises the vector of claim 20.

25. A recombinant host cell which comprises the vector of claim 21.

26. A recombinant host cell which comprises the vector of claim 22.

27. An isolated polynucleotide which encodes the fusion protein of claim 4.

25 28. An isolated subgenomic polynucleotide which encodes the polypeptide of claim 7.

29. A method of screening for agents useful in inhibiting mitogenesis, comprising the steps of:

(a) contacting a cell with a test compound, wherein the cell comprises:

i) a first fusion protein comprising (1) a DNA binding domain and (2) all or a portion of a mammalian  $\gamma$ II-adaptin, wherein the portion is capable of binding to p85 subunit of phosphatidylinositol 3-kinase;

ii) a second fusion protein comprising (1) a transcriptional activating domain and (2) all or a portion of a p85 subunit of phosphatidylinositol 3-kinase, said portion comprising a *Bcr* homology domain of p85 subunit of phosphatidylinositol 3-kinase consisting of amino acids 134-322 as shown in SEQ ID NO: ID NO: 4, wherein the interaction of the first and second fusion proteins reconstitutes a sequence-specific transcriptional activating factor; and

iii) a reporter gene comprising a DNA sequence to which the DNA binding domain of the first fusion protein specifically binds; and

(b) measuring the expression of the reporter gene, wherein a test compound that decreases the expression of the reporter gene is a potential inhibitor of mitogenesis.

40. The method of claim 39 wherein the portion of mammalian  $\gamma$ II-adaptin consists of a contiguous sequence of amino acids selected from the amino acid sequence shown in SEQ ID NO: 1.

41. A method for screening test compounds to identify agents useful for inhibiting mitogenesis, comprising the steps of:

(a) contacting a cell with a test compound, wherein the cell comprises:

i) a first fusion protein comprising (1) a DNA binding domain and (2) all or a portion of a p85 subunit of phosphatidylinositol 3-kinase, said portion comprising a *Bcr* homology domain of p85 subunit of phosphatidylinositol 3-kinase consisting of amino acids 134-322 as shown in SEQ ID NO: ID NO: 4;

ii) a second fusion protein comprising (1) a transcriptional activating domain and (2) all or a portion of a mammalian  $\gamma$ II-adaptin, wherein

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 97/09087

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C12N15/62 C12N15/81 C12N9/12 C12N1/19  
C12Q1/68 C07K14/47 C07K16/18 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ROBINSON M.: "Cloning and expression of y-adaptin, a component of clathrin-coated vesicles associated with the golgi apparatus" JOURNAL OF CELL BIOLOGY, vol. 111, 1990, pages 2319-2326, XP002041671 cited in the application * see the whole document, esp. p.2325, l. par. *	1-42
A	WO 93 21328 A (LUDWIG INST CANCER RES ;HILES IAN D (GB); FRY MICHAEL J (GB); DHAN) 28 October 1993 see the whole document --- -/-	1-42

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*&\* document member of the same patent family

Date of the actual completion of the international search

24 September 1997

Date of mailing of the international search report

07.10.97

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Kania, T



# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/09087

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9321328 A	28-10-93	AU 3901793 A	18-11-93
		EP 0590126 A	06-04-94
		JP 6510207 T	17-11-94
-----			

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☒ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**